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The genetic basis of durum wheat germination and seedling growth under osmotic stress

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Abstract

Durum wheat (*Triticum turgidum* L. var. *durum*) is mainly produced under rainfed but often sub-optimal moisture conditions in the Mediterranean basin. A set of 114 durum wheat recombinant inbred lines (RILs) developed from the cross of cultivars Omrabi5 × Belikh2 were tested for the ability to tolerate moisture deficiency at the germination and early seedling growth stage. The stress was imposed by exposing the germinating grain to 12 % polyethylene glycol. It induced a measurable reduction in root length, shoot length, and the percentage of normal seedlings. The germination and seedling growth of Belikh2 were more strongly inhibited than those of Omrabi5, and both parents were out-performed by > 50 % of the RILs. A quantitative trait locus (QTL) analysis was carried out by first assembling a linkage map from 265 informative microsatellites. Composite interval mapping revealed nine QTL spread over seven chromosomes. Five of these were associated with coleoptile length, and one of the five explained nearly 29 % of the relevant phenotypic variance. The coleoptile length was significantly correlated with the seedling growth, plant height, and thousand kernel mass derived from field-grown plants of the same RIL population.

Additional key words: drought stress, polyethylene glycol, QTL, recombinant inbred lines, seed size, seed vigour, *Triticum durum.*

Introduction

Production of durum wheat, the grain of which is used for pasta, couscous, and bulgur, represents about 6 % of global wheat production (Connell *et al.* 2004). Most of the crop is grown under rainfed, moisture-deficient conditions in the Mediterranean Basin (Maccaferri *et al.* 2008, Habash *et al.* 2009), a region where climate change models are predicting a large increase in mean temperature and a significant loss in precipitation later in the century (Habash *et al.* 2009).

Moisture deficiency is a serious constraint on plant productivity, affecting cellular metabolism in many ways (McDowell *et al.* 2008, Sade *et al.* 2011), with knock-on negative effects on growth and seed yield (Jaleel *et al.* 2009). In wheat, rapid germination, the development of a long coleoptile, and high seedling vigour are important requirements for crop establishment in drought-prone areas (Blum 1996, Rebetzke *et al.* 1999, Rosyara *et al.* 2009). Since the grain size is positively correlated to the seedling root and shoot lengths (Khurana and Singh 2001, Willenborg *et al.* 2005), it is likely to contribute to the formation of an extensive root system in the mature plant (Leishman and Westoby 1994). The extensive roots may enhance the efficiency of water extraction from soils which are not irrigated, however, they may deplete the water content of the soil too rapidly (Palta *et al.* 2011). There is probably some optimum root to shoot ratio which ensures that water uptake is controlled in such a way that sufficient moisture is retained in the soil during the grain filling period (Passioura 1983, Vadez *et al.* 2013).

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Abbreviations: BARC - Beltsville Agricultural Research Centre; GWM - Gatersleben Wheat Microsatellite; H^2 - broad-sense heritability; ICARDA - International Center for Agricultural Research in the Dry Areas; ISTA - International Seed Testing Association; LOD - logarithm of odds; NS - normal seedling, PEG - polyethylene glycol; QTL - quantitative trait locus; RIL - recombinant inbred line; TG - total germinated seedlings; TI - tolerance index; WMC - Wheat Microsatellite Consortium.

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The drought tolerance is variable in most crops, but is challenging to quantify it (Collins *et al.* 2008, Reza *et al.* 2009). Growing plants under controlled conditions in pots addresses some of these issues, but introduces the question of relevance to the field situation. In addition, a space available in most glasshouses or in hydroponic facilities is limited (Blum 1989, Kulkarni and Deshpande 2007, Kato *et al.* 2008). Hydroponics-based experiments allow a rather precise control over the root environment. In the context of assessing drought tolerance, the stress is commonly achieved by the addition of polyethylene glycol (PEG; Michel and Kaufmann 1973, Chazen *et al.* 1995). In barley, a coleoptile growth in the presence of PEG is well correlated with a field-grown flag leaf and grain mass under drought (González and Ayerbe 2011).

Very little is known about the genetic control of durum wheat grain size, germination, and seedling growth under moisture-limiting conditions. Here, these issues were explored by generating a durum wheat mapping population and then subjecting it to a moisture stress by growing the material in a hydroponic system in the presence of PEG. As the same mapping population has also been grown in the field in Syria, an attempt was to relate the hydroponics-derived seedling phenotypic variation to traits, such as time to maturity, plant height, grain yield, and harvest index measured in a field setting typical of the environment where durum wheat is grown.

Materials and methods

Experimental material and measured parameters: A set of 114 recombinant inbred lines (RILs) were developed from a cross between ICARDA cultivar Omrabi5 (selected to combine drought tolerance with yield potential and yield stability) and breeding line Belikh2 with high temperature and salinity tolerance. Parental lines and RILs were multiplied under optimal conditions, in order to maximize yield and grain quality, in Gatersleben (Germany) in 2009. Three replicates of 15 grains per RIL were laid on two layers of moist filter paper (moistened either with distilled water or with 12 %, m/v, PEG 6000) in a covered transparent plastic box and held at a temperature of 21 ± 1 °C in the dark for three days, then provided with a 12-h photoperiod (an irradiance of 75 μmol m^{-2} s^{-1}) for further five days. At the end of this period, the percentage of normal seedlings (%NS) and of total germinated seedlings (%TG, a criterion of full germination with a minimum root length of 2 mm) was calculated. The maximum root length, shoot length, coleoptile length, and root/shoot length ratio (R/S) were also recorded. The root, shoot, and coleoptile length, and R/S tolerance indices (TI) were derived from the ratio between the values in the presence of PEG and in the control treatment. The grain mass, grain area, grain length, and grain width were all measured in 3 replicates of 100 grains using a *MARVIN* seed analyser (*GTA Sensorik*, Neubrandenburg, Germany). Means and standard deviations were calculated in *Microsoft Excel 2010*, and a standard analysis of variance was performed using the *PlabStat* software (Utz 2011). The statistical significance of differences between the control and the PEG treatments was derived from the paired Student's *t*-test, or, where a normality test failed at $P < 0.001$, the Wilcoxon's signed rank test. Broad-sense heritabilities (H^2) were calculated from the estimates of the genetic variances. The Spearman's correlation was used to illustrate relationships between the various traits.

Marker analysis and linkage mapping: Genomic DNA was isolated from leaves using *DNeasy Plant MiniKit* (*Qiagen*, Hilden, Germany). A set of 1 072 microsatellite markers: GWM (Röder *et al.* 1998, Ganal and Röder 2007), BARC (Song *et al.* 2005), and WMC (*IDnagenetics*, Norwich, UK) were applied to the parental DNA to select assays which would be informative in the RIL population. This analyses resulted in a set of 265 assays (159 GWM, 62 BARC, and 44 WMC) marking loci on all 14 linkage groups. The *MAPMAKER v2.0* program (Lander *et al.* 1987) was employed to carry out a linkage analysis, and recombination frequencies were converted into map distances expressed in cM using the Kosambi (1944) mapping function. Linkage groups were assigned using the "two-point/group" command with a LOD threshold of 3.0. Additional markers were added using the "Try" command, and a final marker order within each linkage group was obtained through the use of the three-point linkage analysis "Ripple" command. The chromosome assignment and localization of the centromeres were determined by the reference to extensive microsatellite-based genetic maps of wheat (Röder *et al.* 1998, Song *et al.* 2005).

Quantitative Trait Locus (QTL) analysis: QTL were assigned using a composite interval mapping method (model 6 with forward stepwise regression) implemented in QTL *Cartographer v2.5* (Wang *et al.* 2011). To control the effects of genetic background, five markers, identified by forward regression, were used as co-factors with a window size of 5.0 cMA. LOD score of 3.0 was applied to declare QTL as significant and reinforced by a calculated threshold value by permutation using 1 000 iterations.

Results

The PEG treatment decreased the germination and seedling growth of RILs and parents, including a highly significant reduction ($P < 0.001$) in the root length, shoot length, coleoptile length, and %NS. Resulted TI were 0.49, 0.59, 0.74, and 0.63, respectively (Table 1). In the control treatment, 72 out of 114 RILs produced %NS < 80 %, whereas in response to the PEG treatment, this parameter ranged from 0 to 84 % (Fig. 1). The shoot length, coleoptile length, and %NS were more reduced in the Belikh2 than in Omrabi5 plants, but the percentage of total germinated seedlings (%TG) was higher in Belikh2 than in Omrabi5. The %TG H^2 was 0.51 in the control treatment and 0.64 in the PEG treatment; for the root length, it was 0.71 and 0.66, respectively. The %NS, shoot length, and coleoptile length H^2 values were lower in the PEG-treated seedlings than in the control ones. The performance of both parents, as measured by TI, was inferior to > 50 % of RILs. Further, results of both parental lines were among 50 % of worse performing RILs after the PEG treatment. The grain parameters varied among the RILs and were all significantly correlated to one another ($P < 0.001$). The H^2 for these characters were

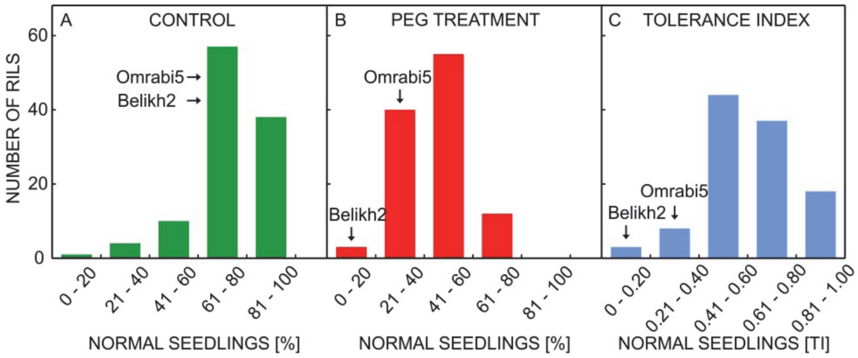

Fig. 1. Growth of Omrabi5 × Belikh2 RILs and parents. Performance under control (non-stressed) conditions are shown in *green*, under the PEG stress in *red,* and tolerance indices (TIs) are shown in *blue*.

Table 1. Grain parameters, germination, and seedling growth of RIL parents Omrabi5 and Belikh2, and the RIL population. Standard deviation (SD), minimum (Min), and maximum (Max) values of the set of RILs are given. Broad-sense heritability (H²) values were estimated from the analysis of variance. PEG - polyethylene glycol, TKM - thousand kernel mass, TI - tolerance index (ratio of the performance under control and PEG treatments). Different letters indicate significant differences between parameters under control and PEG treatments.

	Trait	Omrabi5	Belikh2	RILs mean	SD	min	max	LSD 5 %	H²
Grains	TKM [g]	42.57	39.60	41.63	5.42	25.64	52.87	2.95	0.96
	area [cm²]	20.27	18.77	18.77	1.37	14.73	21.40	0.81	0.95
	length [cm]	3.20	3.23	6.58	0.31	2.60	3.40	0.16	0.94
	width [cm]	6.87	6.30	3.08	0.17	5.70	7.37	0.10	0.96
Control	NS [%]	66.67	68.89	74.37a	14.34	17.78	100.00	25.99	0.58
	TG [%]	91.11	80.00	86.24a	10.09	40.00	100.00	19.28	0.51
	root length [cm]	8.78	8.92	9.03a	2.09	2.00	13.10	3.11	0.71
	shoot length [cm]	6.21	4.37	5.89a	1.81	0.96	10.89	3.18	0.61
	root/shoot	1.41	2.04	1.60a	0.29	1.03	2.46	0.97	0.39
	coleoptile length [cm]	1.78	1.33	1.91a	0.56	0.58	3.81	0.56	0.86
PEG	NS [%]	26.67	6.67	45.46b	14.19	0.00	84.44	37.90	0.26
	TG [%]	68.89	75.56	81.24b	11.42	40.00	100.00	18.81	0.64
	root length [cm]	3.00	3.27	5.06b	1.25	1.73	7.84	2.07	0.66
	shoot length [cm]	1.52	0.68	2.67b	0.95	0.14	4.88	2.22	0.40
	root/shoot	1.97	4.78	2.12b	1.39	1.25	15.90	11.97	0.05
	coleoptile length [cm]	0.92	0.57	1.36b	0.47	0.14	2.81	0.93	0.55
TI	NS [%]	0.40	0.10	0.63	0.22	0.00	1.63	0.56	0.31
	TG [%]	0.76	0.94	0.95	0.12	0.75	1.67	0.25	0.45
	root length	0.34	0.37	0.59	0.21	0.28	1.81	0.26	0.80
	shoot length	0.24	0.16	0.49	0.23	0.04	1.70	0.43	0.53
	root/shoot	1.39	2.34	1.33	0.57	0.82	6.47	6.50	0.01
	coleoptile length	0.52	0.43	0.74	0.24	0.10	1.89	0.53	0.43

all > 0.90 (Table 1). The thousand kernel mass (TKM) and coleoptile length were positively correlated in both the control and PEG treatments (Fig. 2).

The genetic map constructed from the Omrabi5 × Belikh2 RIL population comprised 265 microsatellite loci spanning 2 864 cM (10.8 cM mean inter-marker separation) and divided equally between the two constituent durum wheat sub-genomes (Fig. 3). Individual chromosomes ranged in genetic length from 37 cM (chromosome 1A) to 366 cM (chromosome 7B). Composite interval mapping of the five growth parameters identified the presence of nine QTL on seven different chromosomes. Three of the QTL were expressed only in the control treatment, two only in the PEG treatment, and remaining four were based on TI (Table 2, Fig. 3). The largest individual QTL was for the coleoptile length (LOD 8.4, explaining 28.6 % of the phenotypic variance, R^2). The marker interval 99.8 - 107.6 cM on chromosome 7B harboured QTL for %NS and %TG in the control treatment and a TI for %NS and root length. The other QTL were scattered. Alleles influencing germination and seedling growth positively were shared by the two parents. However, QTL with the highest LOD scores (> 5) came from Omrabi5 and indicated the most tolerance to osmotic stress. The analysis of the four grain parameters revealed eight QTL distributed over three chromosomes with LOD scores varying from 3.4 to 37.7 (Table 2, Fig. 3). A number of loci mapped to chromosome 7B including one for TKM (LOD 11.9, R^2 41.3 %), the grain area (LOD 26.5, R^2 53.1%), and grain length (LOD 37.3, R^2 57.7%). The positive alleles for these loci were all contributed by Omrabi5.

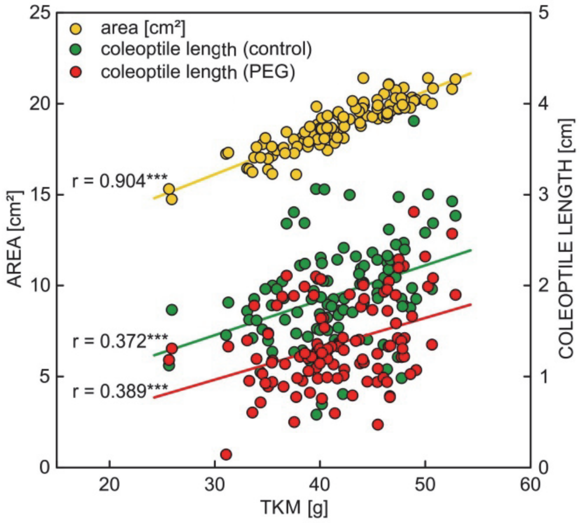

Fig. 2. Relationships between coleoptile lengths and thousand kernel mass (TKM), and grain area and TKM in the RIL population grown in control (non-stressed) and under PEG stress conditions. *** - $P < 0.001$.

Table 2. The QTL analysis of growth parameters and grain characteristics detected in the Omrabi5 × Belikh2 RIL population. QTL declared when LOD exceeded 3. Positive additive effects indicate a contribution by Omrabi5. Chr - chromosome, R^2 - proportion of phenotypic variance explained, TKM - thousand kernel mass.

Trait	Treatment	Chr	Marker interval [cM]	Positions [cM]	LOD	LOD threshold	R² [%]	Additive effects
NS [%]	control	7B	GWM573-GWM1184	107.6	3.24	2.8	10.18	-6.36
	TI	7B	GWM400-GWM1184	99.8	5.61	2.2	21.06	0.13
TG [%]	control	7B	GWM573-GWM1184	107.6	3.01	2.6	9.75	-5.98
Coleoptile length [cm]	control	4B	WMC617-barc199	9.9	8.38	4.0	28.64	0.97
	PEG	1B	GWM947-WMC419	95.6	3.15	3.1	9.97	-0.19
	PEG	4B	GWM894-barc193	0.0	4.24	3.1	14.27	0.23
	TI	3B	WMC56-GWM655	122.3	3.05	2.6	8.98	0.09
	TI	6A	GWM459-GWM356	57.3	4.07	2.6	12.14	-0.10
Root length [cm]	TI	7B	GWM400-GWM540b	99.8	7.94	2.2	28.69	0.15
TKM		7B	GWM16-WMC476	149.6	11.89	2.5	41.29	17.03
Grain area		7B	GWM16-WMC475	149.6	26.53	2.3	53.08	7.89
Grain area		7B	WMC476-GWM297	167.8	3.42	2.3	11.08	-1.24
Grain area		7B	barc255-GWM963b	173.1	21.00	2.3	58.86	7.90
Grain width		2A	GWM122-GWM372	94.9	4.02	2.8	13.34	-0.25
Grain length		4A	GWM181-GWM160	0.0	3.13	2.1	9.10	-0.36
Grain length		7B	GWM16-WMC475	149.6	37.32	2.1	57.69	2.75
Grain length		7B	barc255-GWM963b	173.1	32.03	2.1	64.01	2.79

Discussion

The prediction that the expected outcome of climate change will be an increased occurrence of drought episodes in the Mediterranean basin has prompted much research centred on improving the water use efficiency of

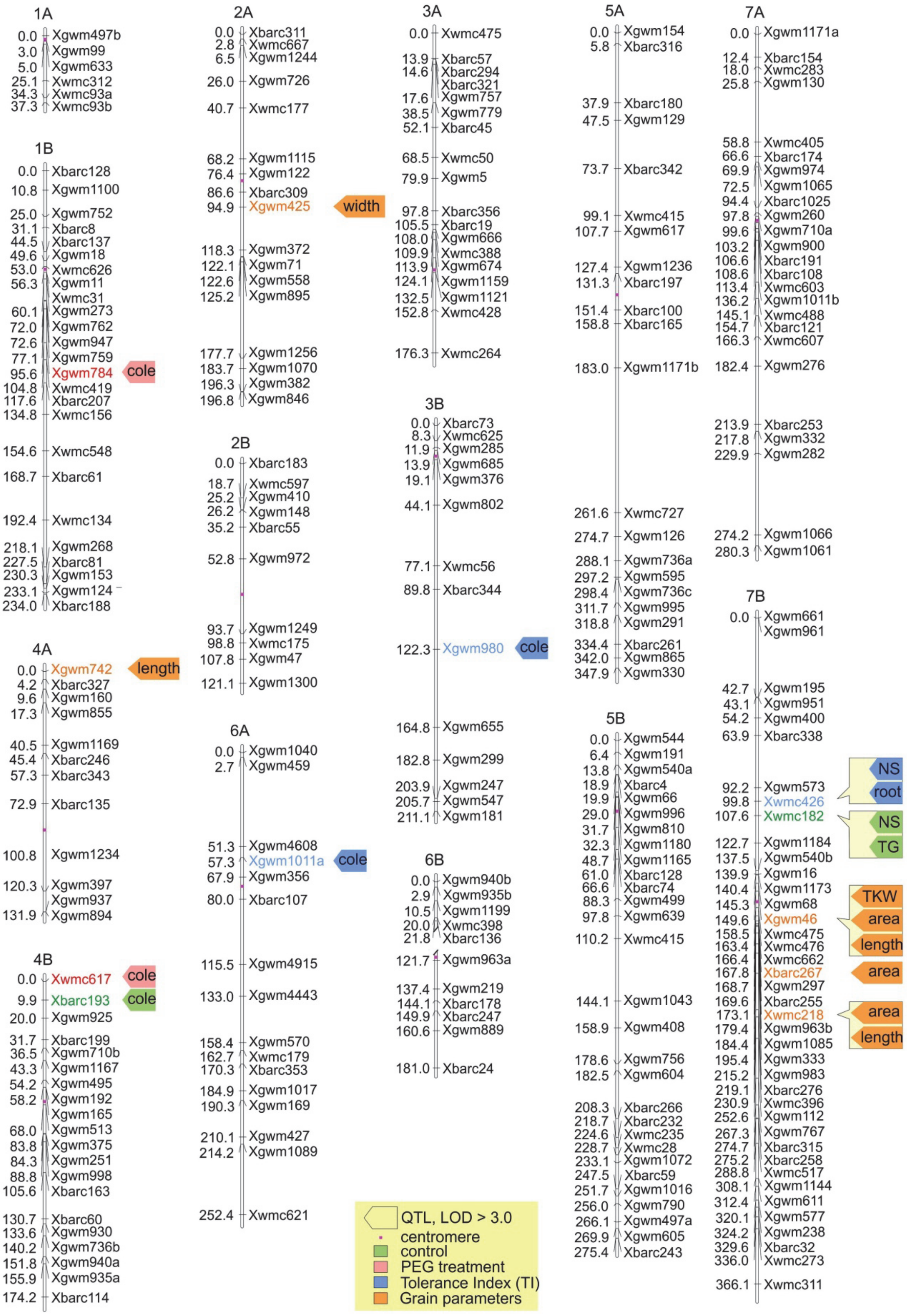

Fig. 3. The linkage map derived from the Omrabi5 × Belikh2 population including QTL locations. QTL expressed under non-stressed conditions are marked in *green*, those under the PEG stress in *red*, and TIs in *blue*. QTL for grain parameters are marked in *orange*. Map distances are shown in cM. QTL declared above a LOD threshold of 3. Cole - coleoptile length, %NS - percentage of normal seedlings, %TG - percentage of fully germinated seedlings.

currently grown crops (Nachit and Elouafi 2004, Tuberosa and Salvi 2006). The present experiments were intended to generate relevant genetic information regarding the durum wheat seedling response to osmotic stress for breeding traits, such as time to maturity, plant height, grain yield, and harvest index in a real field situation.

The exposure to PEG caused a measurable decline in the root, shoot, and coleoptile growths of the RILs. PEG reduces water uptake by the root (Almansouri *et al.* 2001). The reduction in root length induced by PEG was probably due to its osmotic effect, although it has been suggested that PEG can also induce hypoxia under certain conditions (Verslues *et al.* 1998). The effect of the stress was the increased R/S ratio because in the face of a sub-optimal water potential, the growth of the root is generally less sensitive than that of the shoot (Wu and Cosgrove 2000); note that the root length H^2 (unlike the stem length one) was similar in the control and PEG treatments. An effect of reducing overall growth is that the requirement for water is lowered and thus the probability of plant survival is increased, as noted in a study of wild barley (Chen *et al.* 2010).

The effect of seed/grain size on germination is controversial. In general, larger seeds/grains with a bigger reservoir of assimilates enable greater seedling vigour and more rapid stand establishment (Akinci *et al.* 2008). Nevertheless, this association does not always seem to hold (Bouaziz and Hicks 1990, Schutte *et al.* 2008, Schwienbacher *et al.* 2010). Here, neither root growth, shoot growth, nor any of the germination traits correlated with any of the grain parameters under the control conditions. The only significant relationship (albeit with quite a low correlation coefficient) was observed between the coleoptile length and TKM under both the control and PEG treatments (Fig. 2) supporting the outcomes reported by Mian and Nafziger (1994) and Mut *et al.* (2010). The ability of larger grains to produce more vigorous seedlings seems to be an advantage rather under water stress than under well-watered conditions (Mian and Nafziger 1994, Rebetzke *et al.* 1999, Kaydan and Yagmur 2008, Rosyara *et al.* 2009).

Various genetic analyses have been performed in durum wheat, mostly focusing on grain yield components (Blanco *et al.* 2001, Maccaferri *et al.* 2008, Czyczylo-Mysza *et al.* 2011, Golabadi *et al.* 2011). Genetic variation for tolerance to osmotic stress at the seedling stage has been relatively neglected to date, although it has been shown that there are genotype-specific responses to stress (Kato *et al.* 2008, Landjeva *et al.* 2008, Chen *et al.* 2010). The genetic analysis of seedling growth in the Omrabi5 × Belikh2 population revealed nine QTL distributed on seven chromosomes. In bread wheat, the region on chromosome 7B harbouring a number of these loci lies close to the region reported by Zhang *et al.* (2013b) to contain QTL induced by osmotic stress for root number, root and seedling dry masses, and plant height. The coleoptile length QTL on chromosome 6A maps to a region identified by Spielmeyer *et al.* (2007) as harbouring loci involved in coleoptile length and other traits. Major coleoptile length QTL mapping to chromosome 4B lies in a region containing QTL for root length under both well-watered (Liu *et al.* 2013) and water stressed (Zhang *et al.* 2013a,b) conditions. This region also features QTL for carbon isotope discrimination (a surrogate for water use efficiency; Nachit and Elouafi 2004). Both traits, the coleoptile and root lengths on 4B were strongly affected by initial grain quality (Fig. 2).

The quality of the grain is determined by its storage conditions and/or the environment experienced by the mother plant during grain development (Nagel *et al.* 2009, Zhang *et al.* 2013a). The low correlations between seed growth parameters under the control and osmotic stress treatments indicate a different genetic background. However, the source of the positive alleles at the QTL expressed under the PEG treatment (and TI) was mainly from the more drought tolerant parent Omrabi5 which suggests a shared genetic basis for adult plant drought and seedling osmotic stress tolerance. Further, under the osmotic stress, more than 50 % of RILs were more tolerant to the stress than were either Belikh2 or Omrabi5, supporting finally the hypothesis that both the parents could contribute alleles to tolerance against the osmotic stress.

The RIL population has been field tested over two seasons in Syrian drylands (Table S1). Chromosome 4B QTL for coleoptile growth, expressed under both the control and PEG treatments, co-located with QTL for plant height detected in both seasons (Table S2). In the same genomic region, the analysis of a panel of 189 durum wheats grown in 15 environments has revealed marker-trait associations for both plant height and peduncle length (Maccaferri *et al.* 2011). The QTL location overlaps that of the semi-dwarfing gene *Rht-B1b* (Börner *et al.* 1997, Rebetzke *et al.* 2007). However, no relationship was recognized between the coleoptile length and the grain yield in the PEG-stressed RILs implying that the coleoptile length is not the only determinant of the plant vigour contribution to grain yield.

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